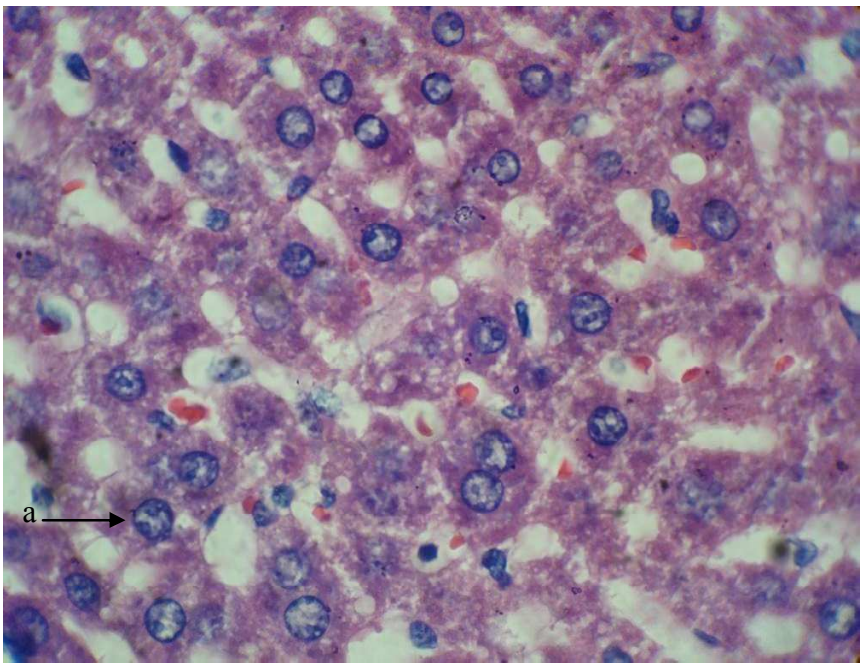
CHAPTER 5

RESULT

5.1 RESULT OF COUNTING TISSUE CHANGES FROM HE STAINING EXAMINATION

Tissue changes were observed from HE staining examination with 1000x magnification in 10 fields from randomized choosing. The examiner counted the number of cells with nucleus changes (i.e, enlarge, karyorhexis, and karyolysis). The results were expressed in percentage of abnormal cells per all cells counted on those fields. From total 30 mice, 30 livers of the mice were made into tissue slides.

A



B

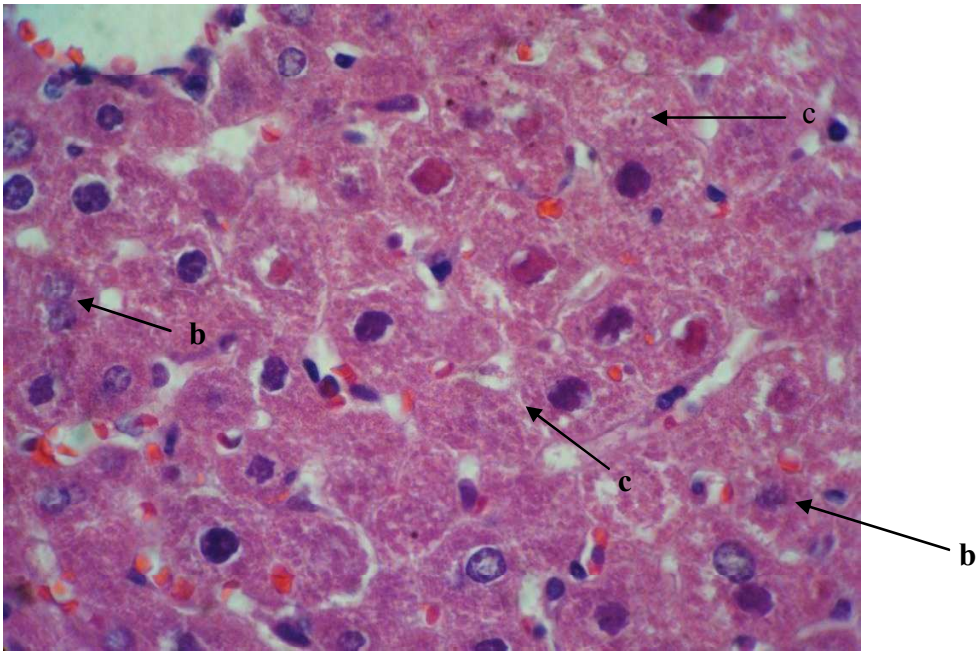


Figure 6. HE staining examination using 1000X magnification from: **A.** control group. The mean of abnormal cells percentage was 17.65%. **a**: enlarge nucleus; **B.** group 3. **b**: karyorhexis; **c:** karyolisis.

Table 4. Percentage of cell changes from HE staining examination

Groups	Mean (%)	Median (%)	SD
Control	17.65	14.35	5.14
Group 1	40.93	40.39	1.71
Group 2	51.94	52.17	1.21
Group 3	61.22	60.79	0.59
Group 4	60.77	63.39	3.60

As shown in Table 4, control group has the smallest percentage of nucleus changes (mean: 17.65%) and group 3 has the highest percentage of nucleus changes (mean: 61.22). Figure 6 shows that box plot median percentage of nucleus liver changes in group 4 is higher than other groups.

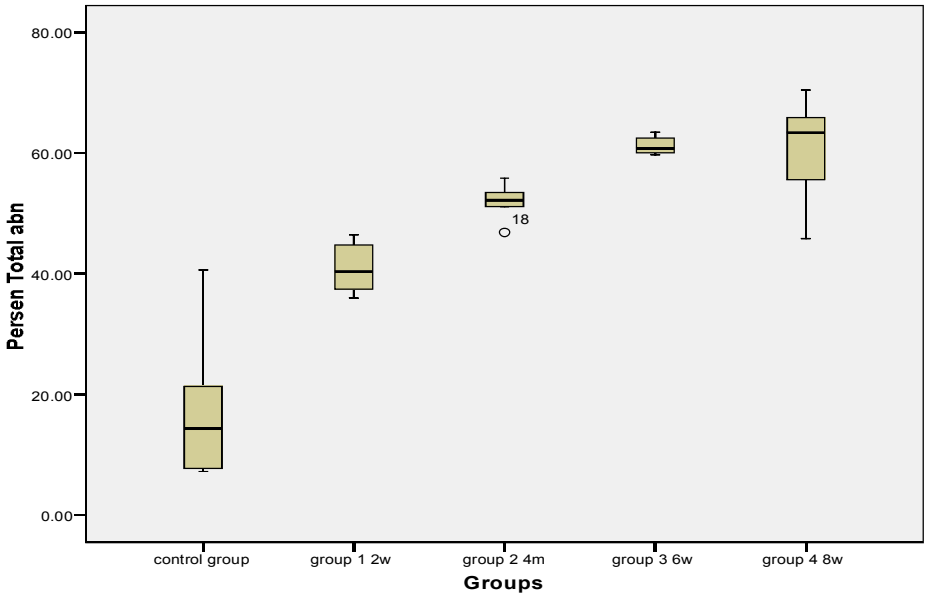


Figure 7. Box plot median percentage of BALB/c Mice liver cells' nucleus changes in control group, group 1, group 2, group 3 and group 4.

The significance of these differences was revealed using statistic test. Using the Shapiro-Wilk test (test full result was attached on the appendix), we found that the data had normal distribution which can be seen in appendix 2, yet

the data variant was not equal, so we could not perform One Way Anova test to know the comparison among all groups. We performed Kruskal-wallis test and the result was statistically significant ($p = 0.00$). Subsequently, we carried out Mann-whitney test as Post Hoc test to know the statistic comparison between each group.

In figure 7, we can see that the percentages of total abnormal cells were positively correlated with the time of exposure, thus, also with the dose of halothane. Interestingly, in the group 4, after six weeks halothane exposure followed by two weeks of free halothane, the mean of abnormal cells were less than group 3 which exposed by halothane for six weeks. However, overall abnormal cells were higher in group 4 which suggest the cells damage still continued regardless the cessation of halothane exposure. The comparison between these two groups was not statistically significant. Thus, I suggest that the liver damage may be irreversible.

Table 5. Comparison between each group (Post Hoc test). It is considered as statistically significant if $P<0.05$

	Control group	Group 1	Group 2	Group 3	Group 4
Control Group	-	0.016*	0.004*	0.004*	0.004*
Group 1	0.016*	-	0.004*	0.004*	0.006*
Group 2	0.004*	0.004*	-	0.004*	0.078
Group 3	0.004*	0.004*	0.004*	-	0.522
Group 4	0.004*	0.006*	0.078	0.522	-

Note: * statistically significant ($P<0.05$)

The differences were statistically significant between each group, except between group 2 and group 4 ($p = 0.078$) as well as between group 3 and group 4 ($P = 0.522$) (table 5).

5.4. RESULT OF IMMUNOHISTOCHEMISTRY EXAMINATION

Expression of cytocrome P450 was observed by immunohistochemistry examination using cytochrome P450 2E1 antibody. Examination and reading of the slides were done by counting the percentage of cytocrome P450 stained brown colour and giving score based on its intensity, 1 for weak staining, 2 for moderate staining and 3 for strong staining. Results were scored by multiplying the percentage of positive cells (P) by the intensity (I). Formula: Q = P x I; Maximum score was 300. The complete results were attached in appendix 3.

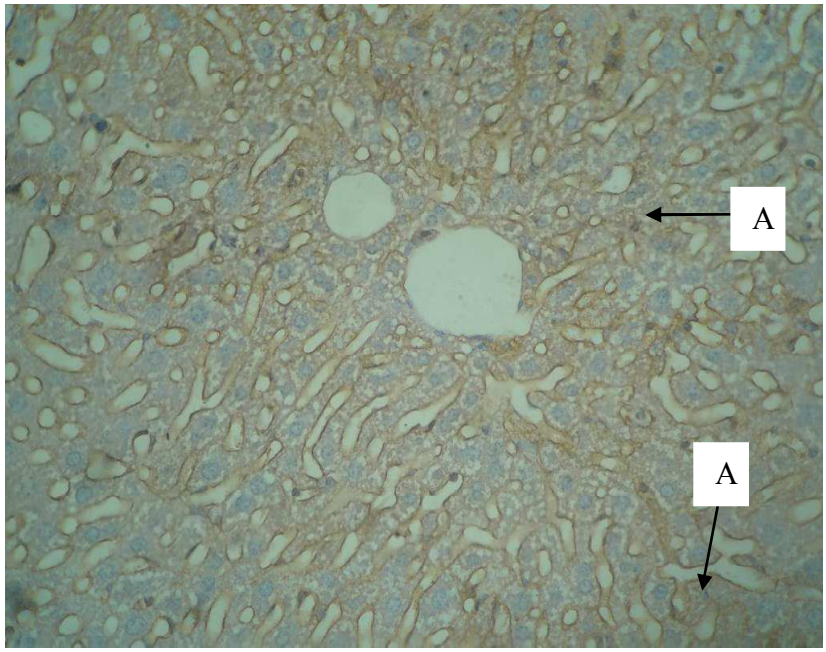


Figure 8. Morphology of P450 expression of the Balb c/ mice liver cells in control group, using 400X magnification. The cells were slightly brown coloured (A).

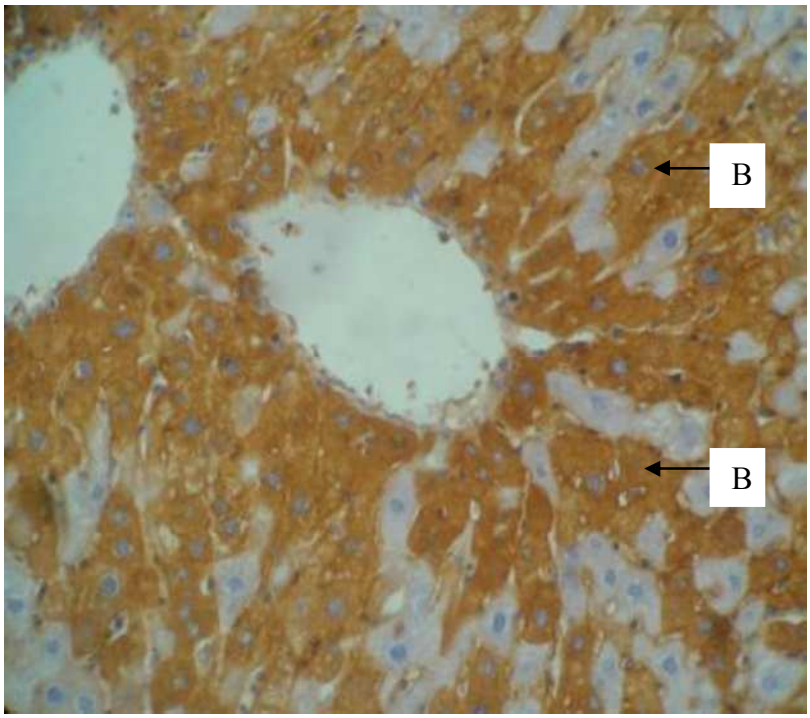


Figure 9. Morphology of P450 expression of the Balb c/ mice liver cells in group 3, using 400X magnification. The cells were intensely brown coloured (B).

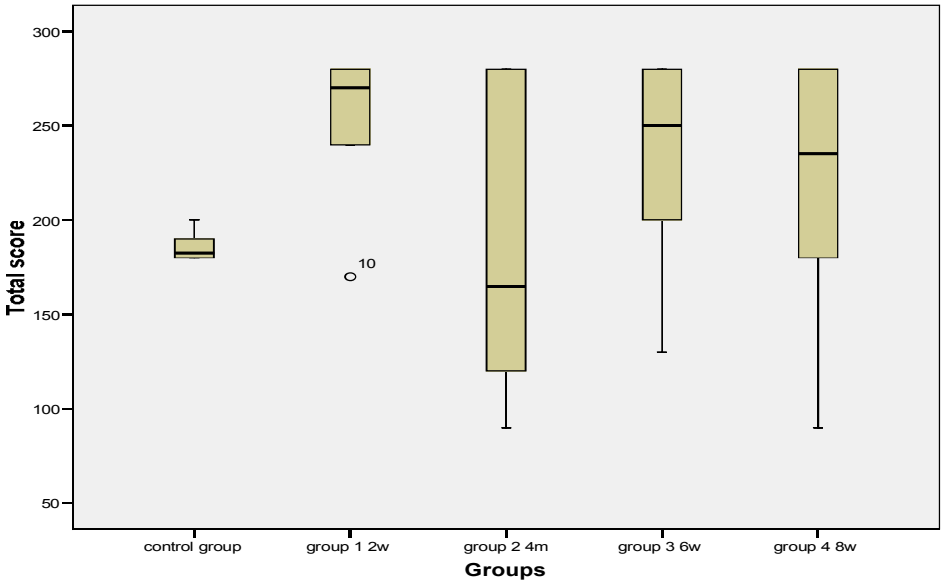


Figure 10. Box plot median percentage of cytochrome P450 expression in control group, group 1, group 2, group 3 and group 4

Table 6. P450 expression scoring from immunohistochemistry staining examination. Results were scored by multiplying the percentage of positive cells (P) by the intensity (I). Formula: Q = P x I; Maximum score is 300.

Groups	Mean	Median	SD
Control	185.83	182.50	3.27
Group 1	251.67	270.00	17.59
Group 2	183.33	165.00	32.93
Group 3	231.67	250.00	24.82
Group 4	216.67	235.00	30.84

The highest median score of cytocrome P450 expression was in group 1 while the lowest median belongs to group 2 (table 6). Shapiro-Wilk test was performed (test result was attached in appendix) and we found that the data distribution was not normal. Therefore, we did data transformation, yet it still had no normal distribution, which can fully seen in appendix 3. To know the comparison of the cytocrome P450 expression among all groups, we used Kruskall-Wallis test. The result from this test was not statistically significant (p =0.266). Thus, we did not conduct Post Hoc test.

Given the results above, there was discrepancy between morphological change and P450 expression. The morphological change already presented earlier than the P450 changes. Therefore, I suggest that this morphological change (nucleus changes) can be used as early marker of liver damage and can be confirmed by liver function test. I also propose that the induction of cytochrome P450 might need longer time to present in the liver. Consequently, in this 8 weeks study the difference of cytochrome P450 expression was not presented in immunohistochemistry examination compare to those changes of cells' nucleus presented in the HE staining examination.